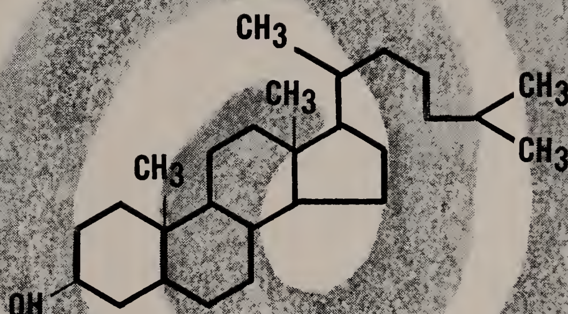
Division of Agricultural Sciences
UNIVERSITY OF CALIFORNIA

CHOLESTEROL



PROBLEMS

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This is a short technical summary of coöperative research, regional project W-44, conducted under the auspices of the Agricultural Experiment Stations of the western states — Arizona, California, Colorado, Hawaii, Idaho, Montana, New Mexico, Oregon, Utah, Washington, and Wyoming—designed to develop additional information on cholesterol metabolism. Specific contributions are designated by name of the state only. The California Agricultural Experiment Station was selected to issue this publication which becomes also a publication of the other western Experiment Stations for distribution under the indicia of each.

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Western Regional Project W-44:

The role of diet in cholesterol metabolism, and biological interrelationships in lipid metabolism of importance to man

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A list of publications and of papers in preparation from the western laboratories is available from members of the W-44 Technical Committee in the various experiment stations.

CHOLESTEROL PROBLEMS

A brief review of the present concepts of cholesterol metabolism and an outline report of the W-44 projects of the Western Regional Experiment Stations and related cholesterol research elsewhere

Cholesterol research has had the advantage of continuing financial support because of wide publicity concerning the relation of cholesterol accumulation to the hardening and thickening of atherosclerotic arteries. The high proportion of deaths attributed to coronary occlusion and atherosclerotic changes in other blood vessels has nevertheless tended to result in pressure to develop practical means for preventing accumulation of cholesterol in blood and tissue, often at the expense of investigations of normal cholesterol metabolism or of the basic mechanisms involved in that accumulation.

Research on cholesterol metabolism during the past fifteen years has been tremendous. A short report such as the one presented here can cover only a small part of it. Good reviews of some of the clinical problems may be found in recent literature, notably the symposia reported in the *American Journal of Clinical Nutrition* (1960, 1961). The monograph edited by R. P. Cook (New York: Academic Press, 1958) deals with the biochemistry of cholesterol.

A brief outline of the comparatively well-established data pertinent to the W-44 project on cholesterol

What cholesterol is

Cholesterol is a white, waxy solid, soluble in fat and fat solvents. Chemically, it is a cyclic alcohol of relatively complex structure and high molecular weight ($C_{27}H_{46}O$).

Where it is found

Cholesterol has attracted the pathologist's attention because it is a constituent of the plaques, or thickened areas, found in the walls of atherosclerotic arteries. It is, however, also found in all tissues of vertebrates. The concentration in muscle is about 0.06 per cent; in liver it varies from about 0.2 to 0.5 per cent when an animal is fed a cholesterol-low diet, but the concentration may increase 10 to 20 times when cholesterol is fed. Human plasma averages between 160 and 250 mg per 100 ml, or approximately 0.16 to 0.25 per cent. Values are usually increased in hypothyroidism, diabetes, and other metabolic disorders. Brain and spinal cord contain about 3 per cent cholesterol; egg yolk, approximately 2 per cent; and butter, 0.3 per cent. Strangely enough, adipose tissue and animal fats, other than wool fat, contain very little. Lard contains less than 0.1 per cent. Cholesterol does not occur in plant tissues nor is it the chief sterol in those of invertebrates, such as crab, shrimp, oyster, etc. Sterols of similar, but not necessarily identical, structure have been found in all species investigated. Some of the related compounds found in plants have been shown to interfere with the absorption of cholesterol from foods, and are, themselves, relatively poorly absorbed.

Its functions

The functions of cholesterol in the animal body are not fully known. Evidence indicates that it is a precursor of steroid hormones elaborated in the adrenals and

the ovaries, and of vitamin D in the skin. As a constituent of cell membranes, cholesterol may affect the ease with which different nutrients enter cells. In nerve tissue it may limit the path of a given impulse. Recent findings indicate that plasma cholesterol may serve as a carrier of essential fatty acids. Plasma and liver cholesterol values drop sharply during some acute infections. White blood cells are very rich in cholesterol (*Oregon*). The above suggest a function in relation to resistance to infection. Apparently, therefore, cholesterol fulfills some useful purposes in the body.

Where it comes from

Cholesterol in animal tissues is derived either from diet or from tissue synthesis. Acetic acid, as well as other fatty acids of low molecular weight, serves as raw material. In other words, it can be made from metabolic breakdown products of carbohydrate, fat, or protein. The synthesis is a complex one which takes place in stages. Some of the more important intermediaries are mevalonic acid, farnesoic acid, squalene, and desmosterol. The liver both synthesizes cholesterol and breaks it down to bile acids, which it excretes. However, most tissues apparently share the liver's capacity to synthesize cholesterol. Cholesterol synthesis has been studied with labeled isotopes, both in the intact animal and with surviving tissue slices. One particularly interesting finding is that liver slices taken following a fast have lost their capacity to make cholesterol from acetate.

How it is absorbed and transported

Cholesterol is fat-soluble and water-insoluble. It is more readily absorbed from the digestive tract when it is fed with fat. It is absorbed via the lymph,

DEFINITION OF TERMS

Atherosclerosis. Thickening of the walls of arteries with accumulation of lipid or fatty material, usually in definite areas or plaques.

Sterol. A general term applied to solid alcohols having a ring structure similar to that of cholesterol. Characteristic sterols are found in the tissues of plants as well as animals. Cholesterol is the important sterol in tissues of vertebrates.

Steroid. Having a structure similar to (but not identical with) a sterol. The term is applied to an important group of hormones secreted by the adrenals, the ovaries, the testes; for example, cortisone, estrogens. The term is also sometimes applied to oxidation products of sterols, such as bile acids.

Isotopes. Different forms of chemical elements. The term is often applied to a form of the element having special properties, such as radioactivity, which permit its measurement when only traces are present.

Coronary occlusion. Blocking of circulation in the coronary artery. Often caused by impaction of a blood clot in an atherosclerotic blood vessel.

Essential fatty acid. One required for normal nutrition and not synthesized in the body. The two most important are linoleic (furnished by vegetable oils) and arachidonic, which is found in lipids from animal sources.

rather than the portal circulation, that is, it is shunted around the liver, as are long-chain fatty acids. Nevertheless, the liver removes excess dietary cholesterol from the bloodstream.

Feeding cholesterol, except in very large amounts, is more likely to result in increased cholesterol of the liver than of plasma. Different species of animals show great differences, however, in their capacity to maintain plasma cholesterol within normal ranges. For example, it is more difficult to increase plasma cholesterol in a rat, by feeding it cholesterol, than in a rabbit. Except during the immediate period of absorption (2 to 4 hours) of a fat- and cholesterol-rich meal (scrambled eggs and brains, for instance), the plasma cholesterol level in man is altered comparatively little as a result of moderate increases in cholesterol intake. It has been shown that cholesterol synthesis, especially in liver slices, is decreased by cholesterol feeding; that is, rate of synthesis is affected by supply. Changes in cholesterol values of any single individual must be interpreted in terms of starting values, time, and age of subject.

Cholesterol in the animal body exists in two forms—"free," and "esterified" (combined with fatty acids). Rapidity of exchange in tissues is apparently increased by esterification. Only in plasma leucocytes (*Oregon*), in adrenal cortex, and in the livers of cholesterol-fed animals, do we find a considerable percentage of the cholesterol esterified. Both free and esterified cholesterol are usually in more or less loose combination with protein—such as the lipoproteins of plasma. In general, a large proportion of the plasma cholesterol is to be found in the heavier lipoprotein aggregates—those containing a higher percentage of protein—while the larger proportion of the true fat or triglyceride is in the lighter lipoprotein aggregates. The presence of a high proportion of certain types of lipoproteins has been considered evidence of susceptibility to atherosclerosis by Gofman and his associates.

Cholesterol and phospholipids

Cholesterol in tissue is nearly always accompanied by phospholipids. These are compounds that can be synthesized in the body. They contain one or two fatty acid units in combination with phosphoric acid and, usually, a nitrogenous base. They are, essentially, molecules which, unlike cholesterol, tend to orient themselves in oil-water boundaries, with the fatty acids toward the oil and the highly polar base toward the aqueous solution. Lecithin, the chief phospholipid of liver, contains the base choline, which is rated as lipotropic, that is, when fed it prevents the accumulation of excess liver fat and cholesterol. Consequently, a great deal of attention has been devoted to cholesterol:lecithin ratios in assessing the effects of diet on tissue composition. Generally, the ratio increases with age or inactivity of a tissue. Phospholipids as well as cholesterol are found in lipoprotein aggregates.

Cholesterol and essential fatty acids

Fatty acids are classified as "saturated," or filled to capacity with hydrogen, and "unsaturated," or having the capacity to take up 2 or more additional hydrogen atoms per molecule. A monounsaturated acid, such as palmitoleic or oleic, has one carbon-carbon double bond ($C=C$), and can take up 2 more hydrogens; a dienoic, such as linoleic, has two carbon-carbon double bonds ($-C=C-\overset{|}{C}-C=C$), and can take up 4 atoms of hydrogen, and so forth. The term "polyunsaturated" is used popularly to designate any fatty acid that has more than one "double bond," or can take up 4 or more hydrogen atoms per molecule.

Certain unsaturated fatty acids cannot be made in the body and must be sup-

plied by diet. Phospholipids and cholesteryl esters carry a large proportion of the essential fatty acids in tissue, especially when the supply in the diet is limited. Linoleic acid (a C_{18} acid with two double bonds) is the chief dietary source of essential fatty acid. There is reason to believe, however, that the "working" essential fatty acid is arachidonic (a C_{20} fatty acid with 4 double bonds), and that it is elaborated, in tissue, from linoleic acid. It is interesting that linoleic acid occurs in comparatively high percentages in vegetable oils and in the adipose tissue fat of animals fed linoleate-rich fat, while arachidonic acid is found only in trace amounts except in cholesteryl ester and phospholipid of animal tissue (*California*). Arachidonic acid is easily oxidized, and foods containing it deteriorate rapidly. The percentages of arachidonic acid in cholesteryl ester vary both with diet and with species of animal. Swell and Treadwell, at George Washington University Medical School, have postulated that an animal's tendency to develop atherosclerosis is inversely proportional to the usual percentage of arachidonic acid in that animal's plasma cholesteryl ester. On the other hand, high percentages of saturated and moneonoic acids in cholesteryl ester have been taken as indications of ease of development of atherosclerotic lesions.

Work partially supported by Western Regional Project W-44

This work has been directed primarily toward increased understanding of the factors influencing metabolism of cholesterol, rather than the pathology of atherosclerosis. While studies with human subjects have been emphasized, certain aspects of the problems, for example, changes in tissue composition, have obviously had to be studied with experimental animals. Cost of human studies has also been a serious limiting factor. Some of the findings from the more productive areas of investigation, both in the Western Region and elsewhere, are outlined below. Stations contributing specific research are indicated in parentheses.

Effect of ethnic origin, climate, and altitude

The Western Region includes peoples of many ethnic groups. Its areas of desert, mountains, and coastal plains were considered to offer excellent opportunities for study of the effects of climate and altitude. Ethnic origin, *per se*, did not appear to influence serum cholesterol values significantly (subjects studied in Hawaii, Thailand, and Manila [*Hawaii*]). The low values observed in Navajo and Papago Indian and Spanish-American children (*Utah*, *Arizona*, and *New Mexico*) may very well have been related to diet. It was interesting that no correlation of serum cholesterol with changes in temperature was found in studies with nurses and postmen in desert heat (*Arizona*). Older women living at altitudes of 7,000 feet (*Wyoming*) were found to have higher serum cholesterol values than did a group of older women at sea level (*California, W-4*).

Regulation of total food intake

The literature indicates that continued gross overfeeding may be conducive to development of coronary lesions, but this result may have been due indirectly to lack of muscular development and the burden of carrying excess fat. Increased caloric intakes compensated for by exercise did not significantly affect the serum cholesterol values in university athletes

(*Utah*) and in postmen (*Arizona*). Numerous data indicate that moderate limitation of food intake and moderate exercise may be expected to be conducive to normal cholesterol metabolism.

Overnight fasting lowered serum cholesterol values in rats. While liver size was decreased, the percentages of liver lipid and cholesterol often increased, possibly because the loss of weight was due to a loss of water and glycogen (*Montana, California*).

A number of investigations have shown that time limitation of access to food ("meal feeding") in experimental animals may, in female rats fed cholesterol, increase the level of plasma cholesterol (*California*). Investigators elsewhere have suggested that "meal feeding" stimulates cholesterol synthesis. The possibility that ingestion of a large meal merely prolongs post-absorptive increases in the serum cholesterol values cannot, however, be ignored. The beneficial effects of more uniform distribution of total food intake throughout the whole day have been emphasized by investigators at Chicago.

Feeding of specific substances

The influence of protein intake on cholesterol metabolism has been widely investigated. The histo-pathologists have found evidence that the initial lesions in arteriosclerosis are in the protein walls of the arteries, and that these lesions may be found even in very young children. These workers have suggested that the subsequent deposition of fatty material is secondary to those early lesions. Accumulating evidence indicates that the quality of protein eaten may be important at all stages of development of lesions. Investigations point to the importance of amino acid balance. High intakes of certain amino acids, such as cystine (*California*), or low intakes of some amino acids, such as threonine (*work in other regions*), have been shown to produce fatty livers in experimental animals. On the other hand, methionine, probably because it is a source of labile methyl, tends to prevent lipid accumulation in liver (*Arizona, California*). Protein intakes that are too low to support normal growth have been shown to produce fatty livers in young rats (*California*). Results favor an adequate, but not excessively high intake of protein of good quality.

Feeding cholesterol has resulted in a decrease in the amount of cholesterol synthesized by experimental animals. Normally, excess cholesterol absorbed from the digestive tract leads to accumulation of cholesteryl ester in the livers of animals rather than to any large increase in plasma cholesterol. However, different species show different responses to cholesterol feeding. Many laboratories elsewhere have fed excess cholesterol with thiouracil (which interferes with thyroid function), and with cholic acid (which increases cholesterol absorption), and have so produced high serum cholesterol values in even the most resistant species of animal, such as the rat. There is some question whether results secured by this procedure are comparable with those observed when cholesterol is fed to man.

The effects of feeding fats of different composition at various levels have been investigated. Fats rich in linoleic acid, such as safflower, corn, cottonseed, soy, and peanut oils, evidently have some effect in lowering serum cholesterol levels in human subjects (*California, Utah*). Feeding these same fats in excessive quantities to laboratory animals resulted in the development of fatty livers (*Utah, Idaho, Montana, California*). When moderate amounts of the highly unsaturated fats were fed with cholesterol, the amounts of cholesteryl ester in the resulting fatty livers were significantly greater than in livers of animals fed otherwise identical diets, which contained butter or hydrogenated shortening or coconut oil (*California*). Coconut oil consists largely of saturated fat, chiefly laurate

and myristate, while butter is rich in saturated acids of very low molecular weight, but also contains a large percentage of oleic acid. Species and age differences in the effects of feeding butter are especially marked.

The polyunsaturated fats combine very readily with oxygen and thus become rancid. Oxidative deterioration may take place during storage, in the digestive tract, or in tissue, following absorption. The presence of such oxidation products is characteristic of aging and atheromatous tissue. The quantities and kinds of natural antioxidants in food fats are consequently important. It is especially interesting that substances closely resembling vitamin E, but not identical with it, have proved the more potent antioxidants outside the body and in the digestive tract, while vitamin E *per se* (α-tocopherol) is the better antioxidant in tissue. The effectiveness of both types of antioxidant may be greatly altered by other substances normally present in foods or tissues, for example, amino acids, enzymes, etc. It is therefore not easy to predict the stability of a polyunsaturated fat. Caution in advising the use of large amounts of highly unsaturated fat in the diet therefore seems desirable. (See review of M. K. Horwitt, *Amer. Jour. Clin. Nutrition*, **8**:451, 1960).

Effect of vitamins and minerals

The effects of vitamin A on serum cholesterol in human subjects have not been consistent, probably because of differences in experimental procedure. In rats given very high dosages of vitamin A with cholesterol, lowering of serum and liver cholesterol was observed (*Idaho*).

Vitamin D effects on cholesterol metabolism were of special interest because of the wide differences in altitude and ultraviolet radiation in the areas covered by the states participating in the W-44 project. However, no significant effects could be demonstrated in the animal study carried out in *Colorado*.

A negative correlation between ascorbic acid intake and mortality from heart disease shown in nutritional status studies with older people (*W-4*) pointed to the desirability of further study of ascorbic acid in relation to fat and cholesterol metabolism. No significant correlations between serum ascorbic acid and cholesterol concentrations were found for six women on a constant diet (*Oregon*); lowering the ascorbic acid intake resulted in a significant decrease in serum cholesterol concentration for one adult woman (*Arizona*), but no consistent change among twelve adolescent girls (*Washington*).

Attention has been drawn to riboflavin since the flavin-containing enzyme systems are important in the intermediary metabolism of fat. Supplementary riboflavin reduced the usual effect of a high fat intake in producing an increased concentration of liver cholesterol in rats, but resulted in higher concentrations of serum cholesterol; increasing fat intakes also tended to decrease the concentration of riboflavin-containing coenzymes in both serum and liver of rats (*Colorado*). No correlation of fasting serum cholesterol to serum riboflavin concentrations was found for six women on controlled diets (*Oregon*). A test dose of riboflavin, which increased concentrations of riboflavin-containing coenzymes in serum, red cells, and white cell-platelet fractions of blood, resulted in no change in serum or red cell cholesterol, and in inconsistent changes in the white cells and platelets of human subjects (*Oregon*).

Rats on biotin-deficient diets lost capacity to incorporate labeled acetate into liver lipids but not into liver glycogen (*California*).

Pyridoxine has been studied as a possible requirement for synthesis of arachidonic acid from linoleic acid (*California*).

Choline, like other sources of labile

methyl, tended to prevent the accumulation of fat and cholesterol in liver (*Montana, California,* and *elsewhere*). In laying hens, choline lowered serum cholesterol concentrations and reduced cholesterol deposits in the aorta (*Arizona*).

Mineral metabolism, especially the metabolism of calcium, magnesium, and iron, has been considered as possibly interrelated with the functioning of cholesterol in the animal body. Higher serum cholesterols were associated with the high hemoglobin values observed at 7,200 feet (*Wyoming*).

Effect of hormones

Deficient thyroid secretion usually results in high serum cholesterol values. Administration of thyroid lowers serum cholesterol. However, studies with normal student nurses, and with postmen, during a hot summer, showed no correlation between the lowered PBI (protein-bound iodine) and basal metabolism and serum cholesterol values (*Arizona*).

Administration of estrogenic hormone to intact and castrate, cholesterol-fed rats increased serum and liver cholesterol values (*California*).

Further work needed

In many cases, a study planned to deal with one problem has inevitably raised additional questions. No attempt has been made in this report to outline the work on methods, which has made it possible to carry out the studies of cholesterol and lipid metabolism. New Mexico, Oregon, Utah, and Hawaii have developed and/or published micro procedures for cholesterol determinations for serum, blood cell fractions, and livers. California has devoted considerable time to chromatographic separation of lipids and fatty acids. Indeed, every laboratory in the Western Region has given much attention to laboratory techniques.

As this report indicates, much work is still to be done before full understanding of the functions of cholesterol and their relationships to the metabolism of fats and other lipids is achieved. Research in the laboratories of the Western Region, as well as in those of other parts of the world, has produced conflicting evidence. Recommendations for very drastic changes in the American diet are hardly warranted by our present knowledge of cholesterol metabolism. However, evidence exists for encouragement of regular and moderate exercise and for the maintenance of a desirable body weight. Also, there is evidence to indicate that moderation in the choice and amount of fat eaten is desirable, just as are moderation in total food intake and maintenance of an adequate and balanced diet.